ANNOTATIONES ZOOLOGICAE JAPONENSES
Volume 30, No. 1—March 1957

Published by the Zoological Society of Japan
Zoological Institute, Tokyo University

The Chromosomes of the Japanese Lancelet, *Branchiostoma belcheri* (Gray), with Special Reference to the Sex-Chromosomes

With 12 Test-figures

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(*Communicated by* S. MAKINO)

The question of the behavior of chromosomes during phylogenesis has been of great interest to cytologists, since chromosomes take the most important role in the hereditary mechanism. The lancelet (*Amphioxus* or *Branchiostoma*) is of special zoological interest because of its phylogenetic relation to the Chordata. This animal has been described as a very primitive chordate having morphological characteristics closely related to vertebrates in many respects. In fact, except for the total lack of any part of the vertebrae and the poor development of the head structure, it is considered in a general way to resemble some ancient ancestor of the chordate animals. According to the phylogenetic conceptions advocated by certain theorists, the lancelet may be a degenerate vertebrate, or a less specialized descendant of an ancestor shared by the vertebrates. In connection with its phylogenetic importance, a knowledge of the chromosomes in the lancelet seems to be of important significance in theoretical considerations associated with evolution. At the present time, however, such knowledge remains in an unsatisfactory state, despite several papers published by classical investigators such as Van der Stricht (1895, 1896), Sobotta (1897) and Cerfontaine (1905a, b). These papers deal with course of ovogenesis or of egg-development in *Amphioxus lanceolatus*. It is desirable to establish, through the application of modern technique, exact knowledge of the chromosomes of this group in order to understand its evolutional development. In response to this need, the present study aimed at obtaining an accurate account of the chromosomes in the Japanese lancelet was undertaken, at the suggestion of Professor Sajiro Makino of Hakkaido University.

Supported by a grant-in-aid from the Scientific Research Fund of the Ministry of Education.

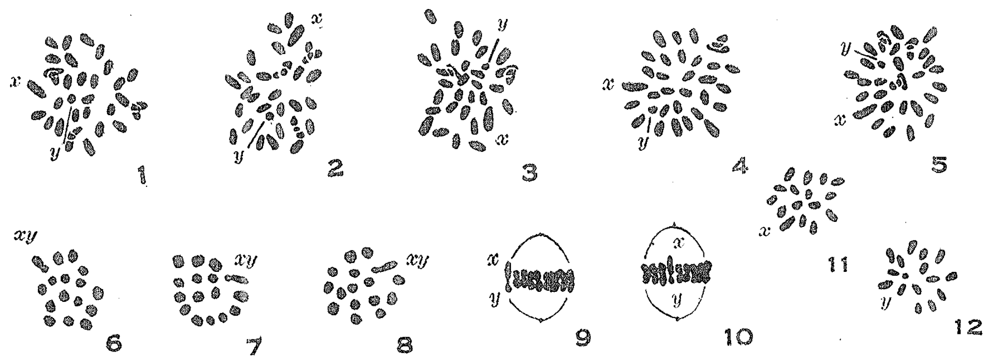
To Professor Makino, the author's cordial thanks are due for his kind guidance and for the improvement of this manuscript. His thanks are also extended to Prof. Tamezo Mori, Prof. Kiyoshi Suzuki and Dr. Osamu Minouchi for their help in various ways, and to the staff of the Saizaki Public Office.

Material and Methods

The lancelet, *Branchiostoma belcheri* Gray, with which the present study is concerned, is a small marine animal of fish-form belonging to the Cephalochordate, with distribution from the middle of Honshu to the south of Kyushu. They are especially abundant in the Seto Inland Sea and near Amakusa, Kyushu. The specimens for this study were collected in the Seto Inland Sea adjoining Saizaki-cho, Hiroshima Prefecture, by the author during March and May 1955, through the courtesy of the Saizaki Public Office staff. The breeding season occurs in May and June. The testes were fixed in Champy's mixture with satisfactory results. The sections were cut 4μ in thickness and stained with Heidenhain's-haematoxylin and light green.

Observations

The Spermatogonial Chromosomes: The germ cells of this species are extremely small in size. A number of dividing figures of the spermatogonia sufficient for the analysis of the morphology and for the determination of the chromosome number were obtained in the sections. From among these, five metaphase figures are presented in Figs. 1–5. A careful chromosome count showed



Figs. 1–12. Chromosomes of the Japanese lancelet (*Branchiostoma belcheri*). 1–5, Spermatogonial metaphases, 32 chromosomes in each. 6–8, Primary spermatocyte metaphases, 16 bivalents in each. 9–10, Side view of the primary spermatocyte spindles, illustrating the XY-complex. 11, Secondary spermatocyte metaphase, including the X. 12, The same having the Y. ×3900.

that the diploid number of this species was constantly 32 in the male. The majority of the diploid members seem to be of the typical rod-type, judging from their short rod-shape and the taper end. The complement contains a pair of elements dissimilar in length, the one being remarkably larger than the other which is extremely small in comparison. The elements other than these two are nearly identical in size. Judging from the remarkable size-difference the two chromosomes seem to represent an XY-pair of the sex-chromosomes, according

to the general rule on the sex-chromosome mechanism. Then the X- and Y-elements are very remarkable in appearance in the male diploid complement on account of their size-difference. Further date for the XY mechanism will be obtained from the observations on the chromosomes in meiotic division.

The Spermatocyte Chromosomes: The chromosomes found in the first meiotic metaphase are very clearly observable because of the reduced number. The primary spermatocyte contains at metaphase 16 well-defined bivalents; they consist of 15 autosomal bivalents and a striking heteromorphic bivalent composed of an X and a Y, as shown in Figures 6 to 8. The XY bivalent always lies in the most peripheral region in the equatorial arrangement. The X conjugates with the Y in the form of an end-to-end association; that is, the rod-shaped X connects in its extremity with the Y of small size in a linear series, as seen in the side view of the spindle (Figs. 9–10). The XY-bivalent stands nearly perpendicular to the equatorial plate at metaphase. The X segregates from the Y in the first division, resulting in the production of two kinds of secondary spermatocytes in respect to the distribution of the X- and Y-elements. Every secondary spermatocyte at metaphase always shows 16 chromosomes. The secondary spermatocytes show a distinction between two sorts, one containing a large X and the other a small Y (Figs. 11–12).

On the basis of the above observations the conclusion was reached that the number of chromosomes in the lancelet is 32 in $2n$ (♂) and 16 in n, and that the chromosome complement consists of 15 pairs of rod-shaped autosomes and an XY pair composed of a large X and a small Y. The X and Y are rather clearly distinguishable from the autosomes because of their characteristic size-difference.

GENERAL CONSIDERATION

The investigation of chromosomes in the Prochordata including the related species dealt with in this study has been carried out by earlier investigators, such as Boveri (1890), Hill (1890), Julin (1893), Bancroft (1899) and Minouchi (1936) in the Tunicata, Van der Stricht (1895, 1896), Sobotta (1897) and Cerfontaine (1905a, b) in the Acrania (cf. the list compiled by Makino, 1956). The majority of these pioneer investigators directed their attention towards the maturating phenomena of the egg cell or the course of egg development, and, consequently, the chromosome studies were of secondary importance to these investigators, who made only an approximate calculation of the chromosome number in these animals. It is, therefore, evident that their results are quite unsatisfactory by present-day standards. Of noticeable importance is the paper published by Minouchi (1931) who studied the chromosomes of *Tethyum plicatum* (Tunicata); he reported the haploid number of 16 in this species, without finding any sexual difference of chromosomes. Recently, Zwillenberg and Zwillenberg (1954) studied the chromosomes of three species of tunicates; they reported that the haploid numbers were 14 in *Aplidium*, 8 in *Pyura* and 9 in *Sidnyum*, and that there occurred no sexual difference of chromosomes in any form. The present report seems to be the first to record the sex-chromosome mechanism in

a species of the Prochordata. The Japanese lancelet, *Branchiostoma belcheri*, showed the diploid number of 32 in the spermatogoninum (♂ $2n$) and the haploid number of 16 in both primary and secondary spermatocytes, with the sex-chromosome mechanism of an XY-type. Obviously, the chromosome number of this species is high in comparison with that of the related form, *Amphioxus lanceolatus*, reported by earlier investigators, Van der Stricht 1895, 1896, Sobotta 1897 and Cerfontaine 1905a, b.

It is evident that all evolutionary changes have had their origin in the chromosomes, and therefore the behavior of chromosomes during phylogensis is of great importance in relation to the evolution of the organism. The phylogenetic relationship of the Prochordata to the Vertebrata has long been a matter of particular interest in taxonomy. Based on the karyological evidence some phylogenetic considerations of the lancelet may be appropriate here.

The relationship of the lancelet to such lower vertebrates as cyclostomes and fishes is to be considered from the karyological viewpoint. As given in the foregoing pages, the chromosomes of the lancelet are isomorphic in complex, i.e., each chromosome is of simple rod-type with a taper end and each one shows no remarkable size-difference, except for the X- and Y-elements with their characteristic shape and size. In general appearance the chromosomes of the lancelet rather resemble those of teleost fishes, since most of them are characterized by a karyotype of isomorphic nature (cf. Nogusa 1953, Makino 1956). Recently the present author had an opportunity to investigate the chromosomes of *Eptatretus burgeri* and *E. okinoseanus*, members of the Cyclostomata (Nogusa unpublished). It was found that the former species had 48 chromosomes, the latter 46. The chromosomes are all of simple rod-type in both species. Whatever the phylogenetic relation may be, these observed facts seem to indicate that the lancelet shows a considerable karyological kinship to some of the cyclostomes. It is then apparent that there is evidence indicating a karyological similarity among the lancelet, some teleosts and cyclostomes. In the light of these facts, it seems probable that there certainly does exist a phylogenetic relationship between the lancelet and lower vertebrates.

A similar relationship is also suggested to occur with respect to the sex-chromosome mechanism. The XY mechanism was established as occurring in two forms of teleost fishes by the present author (Nogusa 1954, 1955), one being *Mogrunda obscura* (Gobiidae) and the other *Cottus pollux* (Cottidae). The present investigation has revealed the occurrence of male heterogamety in the lancelet by demonstrating an XY-mechanism. Here, evidence of a close karyological relationship was obtained again with respect to the sex-chromosome mechanism.

Summary

The chromosomes of the Japanese lancelet, *Branchiostoma belcheri*, were investigated in male germ cells during the course of spermatogenesis. The observations have established the diploid number of 32 in the spermatogonium and the haploid number of 16 in both primary and secondary spermatocytes. The chromosomes are all of simple rod-type. The complement consists of 15

pairs of autosomes of isomorphic nature, and an XY pair composed of a large X and a small Y. The XY pair forms a heteromorphic bivalent in the first meiosis and segregates into the X and Y in the first division, resulting in the production of two sorts of secondary spermatocytes.

On the basis of the karyological evidence, the phylogenetic relationship of the lancelet to lower vertebrates was considered.

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